DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

[0087] As used in this application:

[0088] **"analyte"** means any substance being identified or measured in an analysis, and includes but is not limited to elemental species, element species chelate complexes; cells, viruses, subcellular particles; proteins including more specifically antibodies, immunoglobulins, antigens, ligands, lipoproteins, glycoproteins, peptides, polypeptides; nucleic acids including DNA and RNA; and including peptidic nucleic acids; oligosaccharides, polysaccharides, lipopolysaccharides; cellular metabolites, haptens, hormones, pharmacologically active substances, alkaloids, steroids, vitamins, amino acids and sugars.

[0089] **"analyte complex"** means an analyte bound to other molecules or biologically active materials.

[0090] "**animal**" means all members of the animal kingdom.

[0091] **"aptamer"** means any polynucleotide molecule (for example, DNA or RNA molecule containing natural or synthetic nucleotides) that has the ability to bind other molecules. For example, aptamers have been selected which bind nucleic acids, proteins, small organic components and even entire organisms.

[0092] **"atomic mass spectrometer"** means a mass spectrometer that generates atomic ions, and detects atomic ions based on the mass/charge ratio.

[0093] **"biologically active material(s)"** means any biological substance found in nature or synthetic, and includes but is not limited to cells, viruses, subcellular particles; proteins including more specifically antibodies, immunoglobulins, antigens, lipoproteins, glycoproteins, peptides, polypeptides,

protein complexes (including complexes involving ligands, receptors, or small molecules); nucleic acids including DNA and RNA, aptamers, and including peptidic nucleic acids; oligosaccharides, polysaccharides, lipopolysaccharides, cellular metabolites, haptens, hormones, pharmacologically active substances, alkaloids, steroids, vitamins, amino acids and sugars.

[0094] **"capacitively coupled plasma" (CCP)** means a source of ionization in which a plasma is established by capacitive coupling of radiofrequency energy at atmospheric pressure or at a reduced pressure (typically between 1 and 500 Torr) in a graphite or quartz tube.

[0095] **"capture molecule"** means any molecule capable of binding with an analyte.

[0096] **"competition analyte"** means a purified form of the tagged analyte of interest. The tag may be a radioisotope, fluorescence, enzyme or an element. A competition analyte is used in competition assays in which the analyte of interest in a sample is quantitated by determining the concentration of tagged competition analyte that successfully binds to the analyte binding sites provided in the assay after exposure to the sample containing the analyte of interest.

[0097] **"corona"** means a source of ionization in which a conductor (typically a needle) is provided a voltage relative to a counter electrode surface (typically containing an ion sampling aperture) such that the voltage gradient exceeds a critical value to cause ionization of the surrounding gas, but not sufficient to cause sparking.

[0098] **"cytokine"** means a regulator. In nature, cytokines are usually comprised of soluble proteins and peptides that may be secreted by one cell for the purpose of altering its own function (autocrine effect), those of adjacent cells

(juxtacrine effect), those of near-by cells (paracrine effect), or events occurring in the extracellular environment.

[0099] **"elemental species"** means a molecule containing a metal bound to another atom or group of atoms. For example, selenite (SO_3^{-2}), selenate (SO_4^{-2}), methylselenocysteine and selenomethionine are elemental species of selenium.

[0100] **"directly tagged"** includes any of the methods of tagging described herein, including but not limited to covalently and coordinatively bound transition elements, but excluding tags made up of encapsulated transition elements embedded in latex.

[0101] **"element tagged"** means a molecule tagged with a transition element, including a noble metal or lanthanide.

[0102] **"elemental tag"** means any transition element, including a noble metal or lanthanide, used to tag the biologically active material or analyte.

[0103] **"electrospray"** means a source of ionization in which a liquid sample is nebulized from a tube due to the sufficiently high potential applied, which also provides a charge to the droplet, and in which the resultant charged droplet evaporates and fragments yielding small charged droplets or charged molecular ions.

[0104] **"Fab"** means the antigen binding fragment of an antibody obtained by papain reaction with immunoglobulin.

[0105] **"Fab'"** means the antigen binding fragment of an antibody. Fab' fragments are usually obtained by pepsin reaction with immunoglobulin, followed by cleavage of two disulfide bonds.

[0106] **"Glow discharge" (GD)** means a source of ionization in which a discharge is established in a low pressure gas (typically between 0.01 and 10 Torr),

typically argon, nitrogen or air, by a direct current (or less commonly radiofrequency) potential between electrodes.

[0107] **"graphite furnace"** means a spectrometer system that includes a vaporization and atomization source comprised of a heated graphite tube. Spectroscopic detection of elements within the furnace may be performed by optical absorption or emission, or the sample may be transported from the furnace to a plasma source (e.g. inductively coupled plasma) for excitation and determination by optical or mass spectrometry.

[0108] **"inductively coupled plasma"(ICP)** means a source of atomization and ionization in which a plasma is established in an inert gas (usually argon) by the inductive coupling of radiofrequency energy. The frequency of excitation force is in the MHz range.

[0109] **"lanthanide"** means any element having atomic numbers 58-71. They are also called rare earth elements.

[0110] **"MALDI"** means a source of ionization (Matrix Assisted Laser Desorption Ionization) in which ions are produced from a sample mixed with a matrix (typically analyzed in crystalline form) by exposure to laser irradiation, typically at low pressure

[0111] **"mass spectrometer"** means an instrument for producing ions in a gas and analyzing them according to their mass/charge ratio.

[0112] **"microwave induced plasma" (MIP)** means a source of atomization and ionization in which a plasma is established in an inert gas (typically nitrogen, argon or helium) by the coupling of microwave energy. The frequency of excitation force is in the GHz range.

[0113] **"multiplexing"** means using more than one elemental tag for the simultaneous or sequential detection and measurement of biologically active material.

[0114] **"noble metal"** means any of several metallic elements, the electrochemical potential of which is much more positive than the potential of the standard hydrogen electrode, therefore, an element that resists oxidation. Examples include palladium, silver, iridium, platinum and gold.

[0115] **"optical spectrometer"** means an instrument calibrated to measure either wavelength of light or the refractive index of a prism, and includes atomic emission and atomic absorption spectrometers.

[0116] **"plasma source"** means a source of atoms or atomic ions comprising a hot gas (usually argon) in which there are (approximately) equal numbers of electrons and ions, and in which the Debye length is small relative to the dimensions of the source.

[0117] **"primary biologically active material"** means any molecule that binds to the analyte.

[0118] **"rare earth metals"** means any element having atomic numbers 58-71. The are also called "lanthanides'.

[0119] **"sample"** means any composition of liquid, solid or gas containing or suspected of containing an analyte.

[0120] **"secondary biologically active material"** means any molecule that binds to the analyte or primary biologically active material.

[0121] **"tertiary biologically active material"** means any molecule that binds to the analyte, the primary biologically active material or the secondary biologically active material.

[0122] **"transition element"** means any element having the following atomic numbers, 21-29, 39-47, 57-79, and 89. Transition elements include the rare earth elements, lanthanides and noble metals. (Cotton and Wilkinson, 1972, pages 528-530).

[0123] There are a number of aspects to the present invention.

[0124] The first aspect involves labeling of biologically active material which binds to an analyte in a sample. Most often, the biologically active material would be an immunoglobulin, aptamer or antigen. The element is detected by an atomic mass or optical spectrometer having a source of atoms or atomic ions. Examples 1, 2, 3, 4, 5, 6, 7, 8, 10, 14, 15 and 16 are examples of this aspect of the invention.

[0125] The individual steps involved in this first aspect of the invention are known to those skilled in the art but the coupling of assays with spectrometry is new and inventive. Each of the individual steps is described in Materials and Methods section of this application.

[0126] The benefits of this aspect of the invention are that: (1) it allows for the detection of minute quantities of analyte, (2) it allows for multiplexing, saving time, resources and providing for a better analysis of the sample, (3) the analysis is very rapid as there is no need to wait for enzymatic reactions, and measurement time by ICP-OES/MS is shorter than radiological tag measurement, (4) it has a large dynamic range, (5) radioisotopes are not required, producing a safe work environment and avoids toxic waste, and (6) the reacted complex does not need to be preserved, allowing the use of acidic media to degrade the complex and stabilize the element in solution and thereby increasing the period of storage of the sample before analysis.

[0127] The second aspect involves the determination of elemental species. In cases where mass spectrometry cannot differentiate elemental species, the use of

antibodies or aptamers to detect elemental species coupled with mass spectrometry allows for their differentiation. Examples 11, 12 and 13 describe this aspect of the invention in more detail. In this aspect, as show in Example 13, multiplexing can be used. Again, the benefits of this aspect are that: (1) it allows for the detection of minute quantities of analyte, (2) it allows for multiplexing, (3) the analysis is very

rapid, (4) there is a large dynamic range, (5) one can avoid the use of radioisotopes, (6) the reacted complexes do not need to be preserved, and (7) chromatographic separation is not required, which speeds up and simplifies the analysis.

[0128] The third aspect is the direct labeling of the analyte. The individual steps involved in this third aspect are known to those skilled in the art, but direct labeling coupled with mass spectrometry is new and inventive. Example 17 describes this third aspect of the invention. A variation of this aspect is described in Example 9. Again, the benefits of this third aspect are that: (1) it allows for the detection of minute quantities of analyte, (2) it allows for multiplexing, (3) the analysis is very rapid, (4) there is a large dynamic range, (5) one can avoid the use of radioisotopes, (6) the reacted complexes do not need to be preserved, and (7) chromatographic separation is not required, which speeds up and simplifies the analysis.

[0129] The fourth aspect of the invention is the provision of kits comprising reagents for the detection and measurement of tagged biologically active materials and tagged competition analytes. For example, the kits may include reagents for the detection and measurement of cytokines. Elemental-tagged antibodies, aptamers or cytokines in conjunction with atomic mass or optical spectrometry have never been used to quantitate levels of cytokine in mixed biological samples.

[0130] The benefits of this aspect of the invention are many. The kits allow for an extremely sensitive assay, there is no over-lap in signal, there is a wide dynamic

range (over 8 orders of magnitude), there is a large potential for multiplexing (up to 167 different isotopes are available), radioisotope handling is not required, and the kits can withstand long term storage. These benefits are discussed in detail below. The kits will be described with reference to kits for cytokine analysis, but it is understood

that kits for the analysis by mass or optical spectrometry of any analyte are within the scope of the invention.

[0131] First, the ability to quantitate multiple cytokines simultaneously with an atomic mass or optical spectrometer is not subject to the same problems with over-lapping signal associated with fluorescence.

[0132] Second, due to lower backgrounds and enriched tags, the sensitivity of atomic mass or optical spectrometry in analyzing elemental tags, provides a tool that is more sensitive than existing methods. Both of these features allow the measurement of various cytokines in widely different concentrations which is desirable when assessing immunological phenotypes of patients before and after different therapies.

[0133] Third, being able to determine a plurality of cytokines in a single sample, means that less sample (e.g. blood, mucus, tissue, etc.) is required from each patient, which is always an advantage.

[0134] Fourth, element-tagged affinity assays can draw upon 167 different isotopes for tags which is far more than necessary for quantitating pools of cytokines, and this assay is therefore not limited not by the number tags that can be multiplexed. In comparison current kits manufactured that are designed for cytokine quantitation through use of radiological, fluorescent, or enzymatic reagents are limited to either detecting only one cytokine or several (4-9) over a limited dynamic range with

problems of fluorescence/chemiluminescence signals overlapping and inhibiting sensitivities. Fluorescent systems that claim to multiplex more than ten cytokines are not simultaneous and rely on flow cytometry to separate signals, so that the fluorometric detector is only subjected to two fluorophores at a time. This adds to the time required to perform each assay as measurements are recorded bead by bead.

[0135] Fifth, the kits along with the methods provide a large dynamic range (over 8 orders of magnitude), which is not possible with either fluorescence or chemiluminescence. This feature is a benefit when quantifying multiple cytokines, where it is foreseeable that one cytokine may be expressed at low levels where another may be expressed in much greater concentration.

[0136] Finally, element-tagged affinity assays permit long-term storage prior to analysis. This is very advantageous, as instrument sharing or mechanical problems can create a backlog, which means that plates dependent on fluorescence, or chemiluminescence may suffer from faded signals and therefore inaccurate readings. Long-term storage allows for more flexibility in both time and location as reacted plates can even be shipped dry for elemental analysis at a different location.

[0137] For all aspects of the invention, it is understood that the biologically active material can be added to the analyte, or the analyte can be added to the biologically active material. Further, an analyte complex can be formed, by the binding of molecules to the analyte, as seen in the examples outlined below, in which a series of antibodies (primary, secondary, tertiary) can be conjugated to the analyte.

Tagging Elements

[0138] The choice of the element to be employed in the methods of the present invention is preferably selected on the basis of its natural abundance in the sample matrix under investigation. In order to achieve selectivity, specificity, the ability to

provide reproducible results, and include appropriate standards for accurate quantitation, it is evident that the tagging element should be of low natural abundance. For example, in a preferred embodiment, the rare earth elements or gold can be used as tag materials. Yet, in another embodiment, an unusual isotope composition of the tag can be used in order to distinguish between naturally present elements in the sample and the tag material. In this case non-radioactive isotopes of, for example, iron, potassium, nickel or sodium can be successfully distinguished from naturally abundant isotopes employing the elemental analysis.

[0139] The size of an elemental tag (ratio of atoms which are detectable by means of the elemental analysis to the analyte complex) may be varied in order to produce the most consistent, sensitive and quantitative results for each analyte complex.

[0140] In a preferred embodiment of this invention, several conjugates can be used in one sample simultaneously providing that the tagging material was selected to be different in every assay. In this embodiment the preferred ICP-MS technique is used in order to quantify different tagging elements simultaneously or sequentially depending on the apparatus employed.

[0141] Although many applications of the present method will involve the use of a single elemental tag for each biologically active material (for example, antibody, aptamer or antigen) or analyte, it should be readily appreciated by those skilled in the art that a biologically active material (for example, an antibody, aptamer or antigen) or analyte may be tagged with more than one element. As there are more than 80 naturally occurring elements having more than 250 stable isotopes, there are numerous elements, isotopes and combinations thereof to choose from. For example, there are 20 distinguishable 3-atom tags that may be constructed from only 4 different isotopes, and one million distinguishable 15-atom tags that may be constructed from 10 different

isotopes, or 70-atom tags that may be constructed from 5 different isotopes. Within limits prescribed by the need to have distinguishable tags when in combination, this will allow for simultaneous detection of numerous biologically tagged complexes. It is advantageous if the relative abundance of the tag elements is sufficiently different from

the relative abundance of elements in a given sample under analysis. By "sufficiently different" it is meant that under the methods of the present invention it is possible to detect the target biologically active material (for example, antibody, aptamer or antigen) or analyte over the background elements contained in a sample under analysis. Indeed, it is the difference in inter-elemental ratios of the tagged biologically active material (for example, antibody, antigen or aptamer) or analyte and the sample matrix that can be used advantageously to analyze the sample.

[0142] It is feasible to select elemental tags, which do not produce interfering signals during analysis (i.e. do not have over-lapping signals due to having the same mass). Therefore, two or more analytical determinations can be performed simultaneously in one sample. Moreover, because the elemental tag can be made containing many atoms, measured signal can be greatly amplified.

Detection of Metal Ions and elemental species

[0143] As was indicated above, an important application of the method of the present invention is the detection of metal in samples, such as toxic metals in environmental settings, including organisms, animals, and humans. Preferably, the invention detects metals in environmental settings. However, as is readily apparent to those skilled in the art, the toxicity of metals depends on the oxidation state, and often on the chemical structure of the elemental species. While an elemental detector, such as uses an ICP source, is able to determine the total quantity of an element in a sample it is generally unable to distinguish different species. There is an ongoing attempt to

use different forms of chromatography to pre-separate the sample before the ICP, but this approach has been plagued with concern about the integrity of the sample, i.e., preservation of the oxidation state during sample preparation. The method of the

present invention provides a means by which a long-standing problem of detecting speciation is overcome.

[0144] In a further embodiment of the present invention, there is provided a method of determining the concentration of a metal ion of interest, preferably toxic metals, more preferably in environmental/biological samples, comprising preparing a biologically active material (for example an antibody or aptamer) which is specific to a selected speciation state of a given toxic metal, reacting said antibody with a solution suspected of containing a toxic metal, and detection of the resulting complexes by application of ICP- MS. Methods for the preparation of an antibody which is specific to a selected oxidation state of a given toxic metal are known by those skilled in the art and are described, for examples, in Bosslet *et al.*(1999), Blake *et al.* (1998), and Bordes *et al.* (1999).

[0145] In a further embodiment of the present invention, an element-tagged biologically active material (for example, an element-tagged antibody or aptamer) is added to a sample containing a speciated element. The sample is split into two halves. The first half of the sample is analyzed for total speciated element. In the second half of the sample, the reacted complexes are separated from the unreacted. The tagging element and the speciated element are quantified in the reacted sample. The speciated element is also quantified in the unreacted sample. In this instance, the results will provide complementary data, and the fraction of the specific species in question will be determined.

[0146] As was also indicated above, an important application of the method of the present invention is the detection of elements of tags in samples by means of laser ablation of polyacrylamide gels where tagged molecules are separated by electrophoresis. Optionally, the sample can be run on an electrophoresis gel and then

probed using element tagged biologically active materials, for example antibodies or aptamers. This application can be used in order to analyze biomolecules in gels or membranes rapidly without destroying the sample. Also, by employing microablation it is feasible to distinguish cancerous cells from normal cells on histological section of biopsy samples using element-tagged antibodies specifically attached to the markers of cancerous populations.

[0147] The following section describes the methods and materials required to carry out the following invention.

METHODS AND MATERIALS

ICP-MS Techniques

[0148] Techniques using ICP-MS or OES can be applied for the purposes of this invention.

[0149] For example, in its latest realization it was described in Tanner el al.(2000a), Baranov et al. (1999), Tanner et al. (1999), Tanner et al. (2000b), and Bandura *et al.* (2000). This successful modification of ICP-MS includes the dynamic reaction cell, which is used in order to reduce isobaric interferences in atomic mass spectrometry. Briefly, the ICP-DRC-MS technique comprises a high temperature plasma in which the sample particles are atomized and ionized; vacuum interface which is designed to transport the plasma together with analyte ions from atmospheric pressure to vacuum; ion focusing optics; the dynamic reaction cell for chemical

modification of the ion current and mass analyzing devise (quadrupole, TOF or magnetic sector). The sample is usually introduced to the plasma as a spray of droplets (liquid sample) or flow of particles (laser ablation of solid surfaces).

Sources of atoms and atomic ions

[0150] The source of atoms or atomic ions can be produced from the following sources: inductively coupled plasma (ICP), graphite furnace, microwave induced plasma (MIP), glow discharge (GD), capacitively coupled plasma (CCP), electrospray, MALDI or corona.

Antibody Preparation

[0151] According to a preferred embodiment of the methods of the present invention, elementally tagged antibodies, or antibodies directed to a metal of interest are employed. Antibodies that bind a target of interest can be prepared using techniques known in the art such as those described by Kohler and Milstein (1975), Wakabayashi et al. (1990), Frackelton et al. (1985) and Gillis (1983), which are incorporated herein by reference. (See also Kennett, McKearn, and Bechtol (1980), and Harlow and Lane (1988), which are also incorporated herein by reference).

[0152] Within the context of the present invention, antibodies are understood to include monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, and $F(ab')_2$) and recombinantly produced binding partners. Antibodies are understood to be reactive against the target analyte if they bind to the target with an affinity of greater than or equal to 10^{-6} M.

Aptamer Preparation

[0153] According to a preferred embodiment of the methods of the present invention, elementally tagged aptamers directed to an anlayte are employed. Aptamers

that bind a target of interest can be prepared using techniques known in the art such as those described in Ellington AD and Szostak JW. (1990); Turek C and Gold L (1990);

Robertson DL and Joyce GF (1990); Gold, L, Polisky, B, Uhlenbeck, O, and Yarus, M (1995); Szostak, JW (1995).

Tagging of Biologically Active Materials

[0154] Preferably, the tagging element is in the form of a nanoparticle, which is attached to a biologically active material, such as for example an antibody, without degrading its activity (tagged conjugate). Examples of techniques for coupling elemental tags to biologically active materials are well known to those skilled in the art. For example, Barlett, P. A. *et al.* (1978), describe a metal cluster compound (Au_{11}) having a core of 11 gold atoms with a diameter of 0.8 nm. The metal core of 11 gold atoms in the undecagold metal cluster compound is surrounded by an organic shell of PAr_3 groups. This metal cluster compound has been used to form gold immunoprobes, for example, by conjugating Au_{11} to Fab' antibody fragments as well as other biological compounds.

[0155] Another metal cluster compound which has been used as a probe is ~~Nanogold™~~ NANOGOLD. ~~Nanogold™~~ NANOGOLD has a metal core with 50-70 gold atoms (the exact number not yet being known but believed to be 67 gold atoms) surrounded by a similar shell of organic groups (PAr_3) as undecagold. The metal core of ~~Nanogold™~~ NANOGOLD is 1.4 nm in diameter.

[0156] A more recent description of techniques for the preparation of biological tags, which may be used in the method of the present invention, is found in Hainfeld *et al.* (1996) (US 5,521,289). Briefly Hainfeld *et al.* (1996) describes, among others, thiol gold clusters produced by forming an organic-gold complex by reacting a

compound containing a thiol with gold in solution. A second equivalent is also added of the thiol compound. Finally the gold organic is reduced with $NaBH_4$ or other

reducing agents and organometallic particles are formed. These have the general formula $Au_n R._m R'_l \ldots$, where n, m, and l are integers, R and R' are organic thiols, (e.g., alkyl thiols, aryl thiols, proteins containing thiol, peptides or nucleic acids with thiol, glutathione, cysteine, thioglucose, thiolbenzoic acid, etc.). With two equivalents of organic thiol compound, clusters with gold cores ~1.4 nm are formed with many organics. The organic moiety may then be reacted by known reactions to covalently link this particle to antibodies, lipids, carbohydrates, nucleic acids, or other molecules to form probes. Mixtures of organic thiols may be used to provide mixed functionality to the clusters. These organo-gold clusters are stable to heating at 100 degrees C.

[0157] These organic thiol-gold preparations may also be made using similar processes with alternative metals to gold, e.g., platinum, silver, palladium and other metals, or mixtures of metal ions, e.g., gold and silver, resulting in mixed metal clusters. The metal clusters together with all other components of a sample are readily atomized and ionized in the high temperature ICP for subsequent MS or OES analysis.

Separation Techniques

[0158] According to one embodiment of the present invention, a tagged conjugate may be isolated for analysis by employing a filtration technique. For example, after incubation of an analyte with the tagged conjugate the sample undergoes filtering through a size separating centrifugal filter. Non-reacted tagged antibody together with other components of the sample mixture including non-reacted antigen pass through the filter into the filtrate. Complexes of analyte and antibody conjugate are left on the filter and after washing can be stabilized in acidic solution.

Since the integrity of the sample (i.e. the chemical form) is not important after separation, the separated sample can be acidified/degraded/stabilized (for example in acidic media) and quantitative analysis is preferably carried out using the ICP-MS technique. The optimal concentrations of all reagents for each system should be determined in an initial criss-cross serial dilution experiment and the concentration of reagent being quantitated must lie within the dynamic range of the standard curve. As will be readily apparent to those skilled in the art, other techniques of separation of free substance or non-complexed proteins from complexed substance may be used, for examples, salting out, chromatography, electrophoresis, gel filtration, fractionation, absorption, polyacrylamide gel electrophoresis, agglutination, affinity separations, immunoassays, or combinations thereof.

Kits

[0159] Kits are provided for all aspects of this invention.

[0160] Kits are provided for the first aspect of the invention in which a biologically active material binds to an analyte in a sample. Examples 1, 2, 3, 4, 5, 6, 7, 8, 10, 14, 15 and 16 describe this aspect of the invention. The kits may include 1) tags comprising transition elements; 2) element-tagged biologically active materials (including antibodies, aptamers, antigens, or combinations of the above) or element-tagged competition analytes, 3) solid supports, for example microwell plate or beads and filter-plate, 4) analyte standards (i.e. analytes of known concentration, unlabeled in the sandwich and direct assays and element-labeled in the competition assays), 5) diluent buffers, 6) assay buffers, 7) wash buffers, 8) elution buffers and 9) protocols and instructions to carry out the detection and measurement of an element in a sample.

[0161] Each of these components is discussed below.

[0162] (1) The tags comprising transition elements as described above.

[0163] (2) The element-tagged biologically active material would preferably contain element tags that are biologically inert and uniform in both size (number of atoms) and isotopic purity. Preferably the tagging of the biologically active material involves the covalent attachment of elemental tags to the biologically active material at sites that minimize loss of activity. The element-tagged biologically active material may include any biologically active material, for example, antibodies, aptamers, or antigens.

[0164] In kits designed for competitive assays, competition analytes of interest are tagged with elements. The tags are preferably biologically inert and uniform in both size (number of atoms) and isotopic purity. Preferably they are water soluble, non-toxic, easily separated from a tagged material by known chromatographic or dialysis methods.

[0165] In the preparation of element-tagged biologically active materials or competition analytes, purification steps are required to separate free element tags from tagged biologically active materials or competition analytes. This may be done using size exclusion chromatography, affinity chromatography, filtration, or dialysis. The purity and quantity of the element-tagged biologically active materials or competition analytes can be analyzed through UV spectrophotometry or atomic mass or optical spectrometry. The affinity of the element-tagged biologically active materials and the element tagged-competition analytes to the target molecule is determined by test protocols of element-tagged immunoassay prior to sale of the kit.

[0166] (3) The assay may comprise solid supports such as microwell plates, beads columns, filters, membranes, gels, or sol-gel to support sandwich, direct, or competitive element-tagged affinity assays. The beads may comprise agarose, sepharose, polystyrene, or polymeric microspheres.

[0167] (4) Purified standards are provided in the kit for the analyte quantitation and consist of known concentrations of purified analyte (unlabeled for sandwich or direct assay and element-labeled for competition assay) and enable calibration curves to be prepared. The standards should be free of high mass elements or chelators that would interfere with element analysis and are preferably lyophilized in a sterile buffered protein base with a preservative.

[0168] (5) Diluent (or dilution) buffers may also be provided for dilution of the purified element-tagged biologically active materials, element-tagged competition analyte, standards or the analyte. Diluent buffers are preferably: sterile, free of high mass elements or chelators, non-toxic, and designed to retain solubility, binding affinities, and native forms of element-tagged biologically active materials, element-tagged competition analyte, and the analyte to be analyzed. The buffers may comprise sterile proteinaceous buffers with preservatives and free from high mass elements or chelators. Diluent buffers for aptamers should be DNase and RNase free and may require DNase or RNase inhibitors to prevent aptamer degradation. Suggested dilution factors may also be included.

[0169] (6) The assay buffers are used to pre-treat the support prior to the assay. These buffers will serve to pre-wet, optimize the pH, chemically activate the supports to be used, or any combination of the above. Assay buffers are preferably: sterile, free of high mass elements or chelators, non-toxic, and designed to retain solubility, binding affinities, and native forms of element-tagged biologically active materials and element tagged competition analytes to be analyzed

[0170] (7) The wash buffers are also included in the kit for washing excess unbound element-tagged reagent from the assay. These buffers are preferably: sterile, free of high mass elements or chelators, non-toxic, and designed to retain solubility,

binding affinities, and native forms of element-tagged biologically active materials, element-tagged competition analytes, and the analyte to be analyzed.

[0171] (8) The elution buffer is used to suspend the element tag from the surfaces of microwell plates or beads to allow for introduction into an atomic mass or optical spectrometer and should be free of high mass elements with the exception of an elemental internal standard of known concentration. The tag does not necessarily have to be separated from the biologically active material or the beads. The elution buffer may preferably be an acid that allows complete solubility of the sample (tag, analyte, antigens, proteins, and aptamers) and separation from plate or bead support. Preferably the elution buffer is also spiked with an element or enriched isotope that has not been used as a tag. The spike will allow for monitoring of any instrumental drift. The elution buffer may not be required for microsphere assays, in which case, reacted microspheres may be suspended in wash buffer and introduced directly into the atomic mass or optical spectrometry.

[0172] (9) Instructions or protocols may also be included for conducting the assays according to the methods described in the invention.

[0173] Kits are provided for the second aspect of the invention for the determination of elemental species. Examples 11, 12 and 13 describe this aspect of the invention. The kits may include 1) a biologically active material (for example, an antibody or apatmer) specific to the elemental species, 2) buffers as described above and 3) instructions for carrying out the protocols as described herein.

[0174] Kits are also provided for the third aspect of the invention that involves direct labeling of the analyte. Examples 9 and 17 describe this aspect of the invention. The kits may include 1) a tag comprising a transition element, 2) reagents for tagging the analyte with a transition element as is known to those skilled in the art, 3) reagents

for running a sample containing the tagged analyte on an electrophoresces gel, 4)

buffers as described above and 5) instructions for carrying out the protocols as described herein.

[0175] In light of the present disclosure, those skilled in the art will readily appreciate other methods and applications of the methods of the present invention.

[0176] The examples below are non-limiting and are merely representative of various aspects and features of the present invention.

EXAMPLES

Example 1. ~~Nanogold~~™ NANOGOLD immunoassay

[0177] The following provides an example of the methods of the invention using the ~~Nanogold~~ NANOGOLD-IgG™ (or ~~Nanogold~~ NANOGOLD-Fab'™; Nanoprobes) immunoassay and its protocol. PBS buffer A is prepared as follows: 15mM NaCl; 2mM sodium phosphate, pH 7.4; 1% BSA (bovine serum albumin). All eppendorf tubes, micro-titer plates, and filters to be used subsequently are treated with PBS buffer A for 1 hour at room temperature to block non-specific binding. This treatment will reduce the non-specific interactions that occur between the plastic used and the analyte and ~~Nanogold~~ NANOGOLD-IgG™. Alternatively low retention plastic products can be used (e.g. Axygen tubes). Following this a solution of the analyte (peptide, protein, etc.) in the concentration range of 1000 to 0.5 pg/µl in PBS buffer A is prepared. All dilutions are stored on ice. Subsequently, 100µl of either analyte dilution or PBS buffer A (for controls) is pipetted into the individual wells of the micro-titer plate (or set of eppendorf tubes). The ~~Nanogold~~ NANOGOLD-IgG™ is pre-filtered through 300kDa (MICRCON™ or ~~Centricon~~™ CENTRICON (both registered trademarks of Amicon Corporation)) centrifugal filter devices. Dilutions of

filtered ~~Nanogold~~ NANOGOLD-IgG™ in PBS buffer A are prepared as follows: A 1:50 dilution is produced by adding 60µl of ~~Nanogold~~ NANOGOLD-IgG™

in 2940µl PBS buffer A. A 1:500 dilution is then produced by adding 100µl of 1:50 ~~Nanogold~~ NANOGOLD-IgG™ to 900µl of PBS buffer A. Depending on concentration range of analyte, 100 to 500 µl of 1:500 ~~Nanogold~~ NANOGOLD-IgG™ is then added to the wells of the plate and then incubated for 1-2 hours at room temperature. The total amount of analyte-~~Nanogold~~ NANOGOLD-IgG™ mix is then pipetted into the sample reservoir (upper chamber) of a 300kDa MICROCON™ centrifugal filter device (max volume 2ml). This sample is centrifuged at 14,000g for 15 minutes at room temperature. The assembly is removed from the centrifuge and the vial separated from sample reservoir. The sample reservoir is inverted in a new vial, and spun for 3 minutes at 1000g to transfer the concentrate to a new vial. Finally, a fixed volume of the collected analyte-~~Nanogold~~ NANOGOLD-IgG™ antibody mixture is diluted to 1ml with 10%HCl/1ppbIr for stabilization. Ir provides an internal standard for ICP-MS quantitation and the acid solution is suitable for the elemental analysis. The linearity of the ICP-MS detector response as a function of the concentration of the analyte human IgG is shown in the results presented in Figure 1.

Example 2. Immunoassay, other than ~~Nanogold~~ NANOGOLD-IgG™ and assay with aptamers.

[0178] According to this example, an antibody is tagged with an element (eg. Eu, Ru, etc.) suitable for analysis by ICP-MS and is introduced into a sample containing an analyte which is an antigen of interest (e.g. human blood proteins). The element-tagged antibody reacts specifically to the target analyte. The resulting tagged analyte complex is separated from un-reacted antibody (as in Example 1, 3, 4, 5, 8, 9,

or 10), and the tagged complex is analyzed by ICP-MS. Variations of this example include:

a) Tagging with multiple atoms to amplify the signal and thereby improving detectability.

b) As, a), except the tag contains several isotopes of the same element or different elements, preferably in a non-natural (unusual) distribution, so that the unique isotope distribution is a determinant of the targeted analyte. It is to be recognized that there are more than 80 naturally occurring elements) of which some 60 may have value in this application) having more than 250 stable isotopes. This allows construction of an enormous number of distinguishable tags. For example, there are 20 distinguishable 3-atom tags that may be constructed from only 4 different isotopes, and one million distinguishable 15-atom tags from 10 different isotopes, or 70-atom tags from 5 different isotopes.

c) As in a) and b), but incorporating different antibodies with specificity to different target molecules, to allow simultaneous determination of different target molecules. The number of simultaneous determinations is limited by the number of distinguishable tags in combination (which is fewer than the number of distinguishable tags in isolation as described above).

d) Using aptamers, Fab', Fab groups in place of antibodies. This is useful when the analyte that you are interested in binding or quantitating is too small or too toxic to have an antibody made that will bind efficiently to it.

Example 3. Protein A ~~Sepharose~~ SEPHAROSE (registered trademark of Pharmacia Fine Chemicals Inc) Immunoassay

[0179] The following provides an example of the methods of the invention using the Protein A ~~Sepharose~~ SEPHAROSE CL-4B™ (Pharmacia) immunoassay and its protocol. Either ~~Nanogold~~ NANOGOLD-Fab'™ or another element-labeled Fab' specific to the target analyte (or host species of the secondary antibody) may be used. There are three types of immunoassays that may be used:

a) Direct immunoassay, which would involve trapping the target protein of interest (protein X) by incubating Protein A ~~Sepharose~~ SEPHAROSE CL-4B™ with an excess of antibody specific to the target analyte, washing off the un-reacted antibody, adding the analyte-containing sample, washing off the unbound components, and then exposing the PAS-antibody-protein X complexes to element-labeled, anti-X Fab'. This is also referred to as a sandwich assay.

b) Indirect immunoassay which would involve trapping the target protein of interest (protein X) by incubating Protein A ~~Sepharose~~ SEPHAROSE CL-4B™ with an excess of primary antibody (e.g. polyclonal) specific to the target analyte, washing off the un-reacted primary antibody, adding the analyte-containing sample, washing off the unbound components, and then exposing the PAS-antibody-protein X complexes to a second antibody specific to protein X (e.g. a monoclonal antibody), washing off un-reacted secondary antibody, and then incubating the PAS-antibody-protein X-antibody complexes with an element-labeled, anti-secondary Fab'. Alternatively beads or micro-titer plates covalently bound to anti-protein X antibodies may be used. This is also referred to as an indirect sandwich assay because proteins are anchored to a surface using a capture molecule.

c) Competition immunoassay, which would involve trapping the target protein of interest (protein X) by incubating Protein A ~~Sepharose~~ SEPHAROSE CL-4B™ with an excess of antibody specific to the target analyte, washing off the un-reacted antibody, adding the analyte-containing sample, washing off the unbound components, and then exposing the PAS-antibody-protein X complexes to a known amount of purified element-labeled protein X.

[0180] PBS buffer A is prepared as follows: 150mM NaCl; 20mM phosphate, pH 7.4; 1% BSA (bovine serum albumin). All eppendorf tubes, micro-titer plates, and filters, and slurry of Protein A ~~Sepharose~~ SEPHAROSE CL-4B™ to be used subsequently are treated with PBS buffer A for 1 hour at room temperature to block non-specific binding. This treatment will reduce the non-specific interactions that occur between the plastic used and the analyte and ~~Nanogold~~ NANOGOLD-Fab'™. Alternatively low retention plastic products can be used (e.g. Axygen tubes). Following this a solution of the analyte (peptide, protein, etc.) in the concentration range of 1000 to 0.5 pg/µl in PBS buffer A is prepared. All dilutions are stored on ice. Subsequently, 100ul of either analyte or PBS buffer A (for controls) is pipetted into the individual wells of the micro-titer plate (or set of eppendorf tubes). To remove large unbound gold particles, the ~~Nanogold~~ NANOGOLD-Fab'™ is pre-filtered through 300KDa MICRCON™ (or ~~Centricon~~™ CENTRICON) centifugal filter devices. Dilutions of filtered ~~Nanogold~~ NANOGOLD-Fab'™ in PBS buffer A are prepared as follows: A 1:50 dilution is produced by adding 60µl of ~~Nanogold~~ NANOGOLD-Fab'™ in 2940µl PBS buffer A. A 1:500 dilution is then produced by adding 100µl of 1:50 ~~Nanogold~~ NANOGOLD-Fab'™ to 900µl of PBS buffer A. Depending on concentration range of analyte, 100 to 500 µl of 1:500 ~~Nanogold~~ NANOGOLD-Fab'™ is then added to the wells of the plate and then incubated for 1-2 hours at room temperature. The sample is centrifuged at 14,000rpm for 2 minutes at room

temperature. The beads are washed four times with PBS buffer A. In method b) the additional steps to include consist of incubating the beads (and attached analyte) with unlabeled primary antibody, washing off un-reacted monoclonal antibody, and then incubating the PAS-antibody-protein X-antibody complexes with an element-labeled, anti-X Fab'. Finally, a fixed volume of 10%HCl/1ppbIr is added to each well. Ir provides an internal standard for ICP-MS quantitation and the acid solution is suitable for the elemental analysis.

[0181] Experimental results obtained according to method a) are given in Figures 2 and 3, using human IgG as the analyte with F'ab-Au as the tagged antibody. Figure 2 provides the calibration results over a relatively low concentration range, and Figure 3 over a higher concentration range. Together, the data exhibit greater than 3 orders of magnitude of detector linearity with respect to the analyte concentration.

[0182] This Example also permits multiplexing to be analyzed and can be used to identify protein-protein interactions. In this method, cell lysate is collected and subjected to the method as above where an interaction is suspected between protein A and protein B. In this case the primary antibody would be specific to protein A and an element-labeled Fab' would be specific to protein B. Interactions with multiple other proteins (e.g. protein C and protein D) could be detected at the same time, providing that different elements were used to label anti-Fab' specific to protein C and anti-Fab' specific to protein D.

Example 4. ~~Dynabeads™~~ DYNABEADS (registered trademark of Dynal Inc) Immunoassay

[0183] The following method provides an example of the invention using the ~~Dynabeads™~~ DYNABEADS (Dynal) immunoassay and its protocol. This immunoassay is performed as in Example 3, using ~~Dynabeads™~~ DYNABEADS in place of Protein A ~~Sepharose~~ SEPHAROSE CL-4B™. Instead of centrifuging the

sample, the sample is exposed to a magnetic device (Dynal MPC™ (registered trademark of Dynal Inc)). This draws the beads to the bottom of the wells between and after each wash step. Again, 10%HCl/1ppbIr is added to each well in the final step to provide an internal standard for ICP-MS quantitation and elemental analysis.

In the same manner as described for Example 3, multiplexing and protein-protein interactions can be identified using this method.

Example 5. Method for detection and quantification of endogenous proteins in cultured cells.

[0184] There are two methods by which the discrete changes in the levels of endogenous proteins in culture cells can be measured.

a) Direct immunoassay, in which an antibody specific to the protein of interest is required. This antibody is labeled with an element suitable for analysis by ICP-MS.

b) Indirect immunoassay, in which an antibody (primary antibody) specific to the protein of interest is required. In addition a secondary antibody specific to the primary antibody is labeled with an element suitable for analysis by ICP-MS.

[0185] A mono-layer of attached cultured cells is grown and treated with conditions of interest. The growth media is removed and the cells are washed with 1xPBS three times. PBS is then replaced with ice-cold methanol and the culture dishes are incubated at –20°C for 5 minutes. The methanol is removed and the cells are allowed to dry completely. An assay buffer (e.g. 10% horse serum, 1% BSA, 0.05% Tween-20, 1xPBS) is added to the culture dishes and the dishes are incubated for 1-2 hours at room temperature. In method a) an antibody specific to the protein of interest is labeled with an element, diluted in dilution buffer and added to the culture dishes. The cells are exposed to the antibody mix for 2 hours at room temperature (or 37°C).

The un-reacted primary antibody is washed away with wash assay buffer. During this time, the element-tagged antibody binds the target protein. In method b) the antibody specific to the protein of interest is not labeled and is diluted in dilution buffer and added to the culture dishes. The cells are exposed to the antibody mix for 2 hours at room temperature (or 37°C). In the next step, the un-reacted primary antibody is washed away with wash buffer. In method b) the element-labeled secondary antibody is diluted in assay buffer and applied to the cells. The dishes are incubated for 1-2 hours at room temperature. The un-reacted secondary antibody is then washed away with wash buffer. Finally, in both methods, an acid solution (e.g. concentrated HCl) is added, to release and dissolve the tagging element. The dissolved element in acid is diluted with 10% HCl/1ppb Ir to provide an internal standard. The acid solution containing the tagging element is then analyzed by ICP-MS to quantify the protein of interest.

[0186] Experimental data obtained according to method b) is shown in Figure 4. This data examines the sensitivity of this immunoassay, by comparing the relative amounts of Smad2 in three different cell cultures; COS (Bars 5 and 6), COS transfected with pCMV5B-Smad2 (COS-smad2) (Bars 1 and 2), and C2C12 cells (Bars 3 and 4). COS cells are known to have undetectable levels of Smad2 protein (using Western blot analysis). Conversely, Smad2 is detectable in C2C12 cell lysate and in COS cells that have been transfected with pCMV5B-Smad2. These cell cultures are prepared in 60mm dishes, fixed with methanol, blocked with TBST buffer and then incubated in either the presence (Bars 2, 4, and 6) or absence (Bars 1, 3, and 5) of polyclonal anti-Smad2 antibody (Upstate Biotech). The cells are then incubated with a gold-tagged anti-rabbit antibody (Nanoprobes), dissolved in concentrated HCl, diluted 2 fold in 10%HCl/1ppb Ir and analyzed using the ICP-MS. Each bar is an average of triplicate samples. Bars 1, 3, and 5 reflect negative control cultures not treated with primary antibody (-+). Cultures treated with both primary and secondary

antibodies (++) show that in the two cell cultures that express Smad2, a substantial increment in the signal over the (-+) results indicates the presence of the Smad2 protein. The third culture, COS, which is not expected to express Smad2, shows a signal for the (++) case that is roughly comparable to that of the blank (-+).

Example 6. Method for determination efficiency of cell transfection.

[0187] The effectiveness of the cell culture transfection is determined by first modifying cells to transduce a tail (e.g. FLAG™). This protocol is useful when antibodies against the analyte of interest are not available. In this case, the expression of a recombinant analyte containing recognizable tails such as Flag, HIS (histidine) or GST (glutathione S-transferase) are particularly useful as antibodies against these moieties are readily available. As in Example 5, there are two methods by which the analyte of interest can be detected (directly and indirectly).

a) Direct immunoassay, in which an antibody specific to the tail is required. This antibody is labeled with an element suitable for analysis by ICP-MS.

b) Indirect immunoassay, in which an antibody (primary antibody) specific to the tail is required. In addition a secondary antibody specific to the primary antibody is labeled with an element suitable for analysis by ICP-MS.

[0188] Between 1-3 days after transfection of the cells, the growth media (typically 10%FBS, depending on cell-type) is removed and the mono-layer of attached cells are washed with 1xPBS three times. PBS is replaced with ice-cold methanol and the culture dishes are incubated at –20°C for 5 minutes. The methanol is removed and the cells are allowed to dry out completely. An assay buffer (e.g. 10% horse serum, 1% BSA, 0.05% Tween-20, 1xPBS) is added to the culture dishes and the dishes are incubated for 1-2 hours at room temperature. In method a) an antibody specific to the tail is produced and labeled with an element that is suitable for analyzing with the ICP-

MS. The antibody is diluted in dilution buffer and added to the culture dishes. The cells are exposed to the antibody mix for 2 hours at room temperature (or 37°C). During this time, the element-tagged antibody binds the target protein through its tail. In method b) the antibody specific to the protein of interest is not labeled. In both cases, the un-reacted primary antibody is washed away with wash buffer. Then, in method b) the element-labeled secondary antibody is diluted in dilution buffer and applied to the cells. The dishes are incubated for 1-2 hours at room temperature. The un-reacted secondary antibody is washed away with wash buffer. Finally, in both methods, an acid solution (e.g. concentrated HCl) is added, to release and dissolve the tagging element. The dissolved element in acid is diluted with 10% HCl/1ppb Ir to provide an internal standard. The acid solution containing the tagging element is analyzed by ICP-MS to quantify the efficiency of the transfection. Culture dishes containing non-transfected cells cultured at the same time can be used as a negative control

[0189] An alternate variation of this Example involves using a 6xHIS-tagged construct™ (Invitrogen), where there is no need for analyte-specific antibodies. Cells transfected with 6xHIS-tagged constructs are fixed with methanol, blocked with the assay buffer and incubated for 2 hours with a solution containing nickel (e.g. Ni-NTA™; Qiagen). The cells are washed to remove free nickel, degraded in acid degraded, and analyzed using ICP-MS for nickel content.

Example 7. Reporter assay

[0190] In the study of transcription factors, it is necessary to quantitate the levels of transcription. There are two methods by which discrete changes in the levels of transcription activity on a specific promoter (or enhancer elements) can be measured. Cultured cells are transfected with expression plasmids of interest along with equal amounts of plasmid containing the promoter of interest linked to a reporter

gene (e.g. GFP). As in Example 5 there are two methods by which the analyte of interest can be detected (directly and indirectly).

a) Direct immunoassay, in which an antibody specific to the reporter is required. This antibody is labeled with an element suitable for analysis by ICP-MS.

b) Indirect immunoassay, in which an antibody (primary antibody) specific to the reporter is required. In addition a secondary antibody specific to the primary antibody is labeled with an element suitable for analysis by ICP-MS.

[0191] Cultured cells are grown and transfected with conditions of interest. Upon analysis, the growth media is removed and the cells are washed with 1xPBS three times. PBS is replaced with ice-cold methanol and the culture dishes are incubated at –20°C for 5 minutes. The methanol is removed and the cells are allowed to dry out completely. An assay buffer (e.g. 10% horse serum, 1% BSA, 0.05% Tween-20, 1xPBS) is added to the culture dishes and the dishes are incubated for 1-2 hours at room temperature. In method a) an antibody specific to the reporter is labeled with an element, diluted in dilution buffer and added to the culture dishes. The cells are exposed to the antibody mix for 2 hours at room temperature (or 37°C). During this time, the element-tagged antibody will bind the reporter. In method b) the antibody specific to the reporter is not labeled. In both cases, the un-reacted antibody is then washed away with wash buffer. In method b) the element-labeled secondary antibody is diluted in dilution buffer and applied to the cells. The dishes are incubated for 1-2 hours at room temperature. The un-reacted secondary antibody is then washed away with wash buffer. Finally, in both methods, an acid solution (e.g. concentrated HCl) is added, to release and dissolve the tagging element. The dissolved element in acid is diluted with 10% HCl/1ppb Ir to provide an internal standard. The acid solution containing the tagging element is analyzed by ICP-MS to quantify the protein of interest.

Example 8. Detection of proteins after electrophoresis using tagged antibodies.

[0192] A sample of proteins is diluted in 2xSDS sample buffer (1% SDS, 2% glycerol, 100mM Tris, pH6.8, 5% β-mercaptoethanol, 1% DTT, 1% PMSF, 0.2% leupeptin, 0.2% pepstatin) and exposed to electrophoresis on a 2-D or polyacrylamide gel (SDS-PAGE or N-PAGE) to separate the proteins. The proteins from the gel are transferred to nitrocellulose using a semi-dry electrophoretic transfer apparatus (or equivalent). The nitrocellulose is blocked for 1 hour at room temperature using a assay buffer (e.g. 5% milk in 1xPBS). An element-tagged antibody that recognizes the target protein is added to assay buffer and the nitrocellulose blot is exposed to the antibody-containing buffer for 2 hours at room temperature. Alternatively an un-labeled primary antibody that recognizes the target protein is used to bind the target protein, followed by washes with wash buffer, and then probing with a secondary anti-primary antibody that is labeled with an element. The nitrocellulose blot is washed three times with wash buffer (0.2%NP40 in 1xPBS). The protein in question is analyzed and quantified by laser ablation.

Example 9. Detections of proteins after modification with 6xHIS-tag™ (Invitrogen) and separation by electrophoresis.

[0193] This Example is similar to Example 8; however, the proteins in the sample are modified prior to electrophoresis so that they have an affinity for an element (e.g. the 6xHIS modification yields affinity to Nickel). The gel or blotting paper containing the separated proteins is washed with a solution containing an element (e.g. Ni) that is bound by the protein modification. The gel or blotting paper is analyzed by laser ablation (or direct excisions and elutions) and ICP-MS.

Example 10. Size Exclusion Gel Filtration Immunoassay

[0194] In this example, ICP-MS is used to detect the presence of a specific analyte. Accordingly, an antibody is tagged with an element (eg. Au, Eu, Ru, etc.) and is introduced into a sample containing the analyte of interest. The elemental-tagged antibody reacts specifically to the target analyte. The resulting tagged analyte complex is separated from un-reacted antibody using gel filtration (e.g. HiPrep Sephacryl™; Pharmacia) in a running buffer containing 1ppbIr. The eluate is collected in 0.5ml increments into a 96 well plate, diluted in acid, and analyzed by ICP-MS.

[0195] Experimental results obtained according to this method for IgG analyte using Fab'-Au antibody are shown in Figure 5. In this experiment an IgG analyte is incubated with an excess of Fab'-Au. The sample is run through a sephacryl S-200 column at a flow rate of 0.5ml/min, using a running buffer of 0.15M NaCl, 0.02M phosphate, pH 7.4, 1ppb Ir. The figure provides the detector response as a function of elution time (eluate number). The first peak observed (the heavier molecular weight) corresponds to the reacted complex, having an expected molecular weight of about 235 kDa. The second peak corresponds to the unreacted tagged antibody having an expected molecular weight of about 85 kDa.

Example 11. Detection and quantitation of elemental species.

[0196] In this example, ICP mass spectrometry is used to measure a quantity of metal identified by an antibody which is specific for a given molecular form or species of a given metal. A solution containing the analyte is then incubated with an antibody, which is specific for the molecular form of the given metal. This solution is treated to separate antibody-metal species complexes from un-reacted antibody and the remainder of components in the sample, although it is important only that species of the given metal other than the species of interest be removed from the sample.

[0197] Preferably, the antibody exhibits little or no ability to bind to species other than the species of interest and exhibits a tight and specific binding of the metal species which is to be measured. Preferably, this binding affinity shows an equilibrium dissociation constant (K_D) on the order of 10^{-9} to 10^{-8}M. The antibody used in such assays also is able to resist interference from other components contained in the sample, which is being assayed. The solution containing the antibody-metalspecies complexes is subject to standard ICP-MS/OES analysis. This approach removes the necessity for a chromatographic pre-separation and consequently improves the sample integrity. It also allows for simultaneous measurement of several elemental species, the method being limited only by the number of antibodies introduced to the sample.

Example 12. Detection and quantitation of elemental species using tagged antibodies.

[0198] According to this Example, as in Example 11, antibodies specific for metal-species are raised according to methods well known to those skilled in the art. The difference in this Example is the antibody is tagged with multiple atoms of a given tagging isotope, or a stoichiometric mixture of isotopic tags. This has two potential advantages. First, in the event that the target metal element is subject to interference in analysis through typical ICP-based interferences (for example argide ion isobaric interferences) tagging the antibody with a normally non-interfered tag allows for interference-free determination, resulting in improved detectablity. Secondly, specific tags for various species of the same target element allows simultaneous measurement of various species (which would not be provided if the elemental tag were the innate target element itself, since the presence of that element in the spectrum would indicate only that one or more of the target species is present). A further advantage according to this approach is that tagging with multiple atoms of

the same isotope allows for signal amplification proportional to the number of atoms of the same tagging isotope.

Example 13. Simultaneous detection of numerous elemental species in a sample using tagged or untagged antibodies.

[0199] According to this example, as in Example 11 and 12, antibodies specific for metalspecies are raised according to methods well known to those skilled in the art. The difference in this Example is that two or more antibodies specific to different elemental species are incorporated, to allow for the simultaneous determination of different speciations of the same or different elements (where each element is differentially tagged).

Example 14. Immunoassay to detect Bovine Spongiform Encephalopathy (BSE) in animal products.

[0200] The methods of Examples 1, 2, 3, 4, 5, 8, and/or 10 are employed to detect BSE in animal products. There are several monoclonal antibodies (15B3, Korth *et al.*, 1997; KG9, Laffling *et al.*, 2001; Bio-Rad Laboratories) that have been produced that target the prion protein PrP thought to be the infectious component responsible for the illness. Monoclonal antibodies specific to PrP are labeled with an element (eg. Au, Eu, Ru, etc.) and used in immunoassays described in either Example 1, 2, 3, 4, 5, 8, and/or 10. Similar products known to be free of BSE would be used as a negative control. In a similar manner other diseases detected for by antibody can be screened for (e.g. HIV, HTLV, Rabies, etc.).

Example 15. Immunoassay to detect ischemic markers in patients believed to have suffered a heart attack

[0201] The methods of Examples 1, 2, 3, 4, 5, 8, and/or 10 are employed to simultaneously detect multiple ischemic markers in human samples. Candidate markers

include: CK-MB, myoglobin, Troponin I, hsp70, BCL2, Bax, IGF, TNFα, angiostatin II.

Example 16. Method for Drug Discovery

[0202] In order to aid in drug discovery, animal cells or animal receptors are placed in multi-well plates. The molecule of interest is added (i.e. potential drug), as well as element-tagged antibody (or element tagged ligand) that recognizes the receptor. The potential drug is in competition with the antibody for adhesion to the receptor. Unbound antibody is washed away, and the amount of bound antibody is determined by ICP-MS. This is inversely proportional to the effectiveness of the potential drug to recognize the receptor. If each well is provided with differently labeled antibodies, then by combining the contents of the wells, one can simultaneously assess the effectiveness of various drugs, or drug compositions by deconvoluting the resultant data. Likewise, differently labeled antibodies for the same analyte can be produced and placed in corresponding wells of different plate (i.e. 10 differently labeled version of the antibody, each one placed in well 1, 1 in 10 plates). The plate contents are combined, the reacted antibodies are separated and analyzed simultaneously, with de-convolution to determine the analyte concentration in the corresponding well of each plate.

Example 17. Detection of tagged proteins using 2D gel and mass spectrometry.

[0203] In this example, the ICP-DRC-MS technique is used in conjunction with the laser ablation of polyacrylamide gels containing proteins tagged by iron. It is well known that ArN^+ and ArO^+ interfere with $^{54}Fe^+$ and $^{56}Fe^+$, respectively. To facilitate the method described in Example 9, it is essential to remove isobaric poly-atomic interferences from the iron isotopes. For example, the ratio of the mass spectrometric signals at m/z=54:m/z=56 (where m/z indicates the mass-to-charge ratio of the ion) measured directly by ablation of the polyacrylamide gel containing a protein

band tagged by iron was found to be 1.14 (whereas the expected value, based on the

natural abundance of the iron isotopes, is 0.063). Utilizing ammonia as a reaction gas in the DRC environment, it is possible to eliminate ArN^+ and ArO^+ interferences by charge transfer reaction. This approach yielded the m/z=54:m/z=56 ratio that approximated the expected $^{54}Fe^+/^{56}Fe^+$ isotope ratio, by which agreement the determination of the tag iron can be confirmed. In addition, the precision of this measurement is significantly improved due to partial temporal equilibration of ions in the gaseous media of the reaction cell (see Bandura, D.R., *et al.* 2000).

Example 18. Detection of a protein using an element-tagged aptamer

[0204] In this example, an element-tagged aptamer is used to detect and measure a protein in a sample.

[0205] Aptamers that bind a protein can be prepared using techniques known in the art such as those described in Ellington AD and Szostak JW. (1990); Turek C and Gold L (1990); Robertson DL and Joyce GF (1990); Gold, L, Polisky, B, Uhlenbeck, O, and Yarus, M (1995); Szostak, JW (1995).

[0206] Aptamers may be labeled as is known to those skilled in the art.

[0207] A solution of the protein in the concentration range of 1000 to 0.5pg/μl in PBS buffer A is prepared. All dilutions are stored on ice. Subsequently, 100μl of either protein dilution or PBS Buffer A (for controls) is pipetted into individual wells of a micro-titre plate (or eppendorf tubes). The element-tagged aptamer is pre-filtered through 300kDa centrifugal filter devices, such as MICRON™ (registered trademark of Amicon Corporation) or ~~Centricon™~~ CENTRICON). Serial dilutions of the tagged aptamer are prepared as is known to those skilled in the art. Depending on the concentration range of the protein, 100 to 500μl of the tagged aptamer is added to the

wells or tubes and incubated for 1-2 hours at room temperature. Following incubation, the combined sample is filtered in a centrifugal

filter device as described above, for 15 minutes at 14,000g at room temperature. The sample reservoir is inverted in a new vial and spun for 3 minutes at 1000g to transfer the concentrate to a new vial. A fixed volume of the collected protein-aptamer mixture is diluted to 1ml with 10%HCl/1ppbIr for stabilization and analyzed in a mass spectrometer.

Example 19. Preparation of kits comprising reagents for cytokine analysis

[0208] In this example, assay kits are prepared for the purpose of detecting and measuring either a single cytokine or multiple cytokines simultaneously using an atomic mass or optical spectrometer. The kits may comprise reagents for (a) direct affinity assays, (2) sandwich affinity assays, (3) competitive affinity assays, or any other assays that utilize element-tagged biologically active materials or element-tagged analytes that are detected by an atomic mass or optical spectrometer. The element-tagged affinity assays involve three basic steps. First the target cytokine(s) from a sample (for example, EDTA-plasma) is bound to supports such as microwell plates, beads or microspheres. A solid support is not required if separation of reaction products (ie. bound tagged biologically active material from unbound tagged biologically active material) is achieved by chromatography (eg. size exclusion gel filtration or centrifugal filtration) instead of by washing. This may be done using affinity, ionic or covalent bonding. Second, the target cytokine(s) is either complexed with an element-tagged biologically active material (for example, an element-tagged aptamer or antibody) or in the case of competitive assay, element-tagged cytokines are added to bind the remaining surface attachment sites. Third, after washing, the

amount of element tag that has been complexed is measured using an atomic mass or optical spectrometer.

1) Preparation of the element-tagged biologically active materials (for example, antibodies, aptamers) and element-tagged competition analytes (for example, element-tagged cytokines) for the kit. For direct or sandwich affinity assays, detection of biologically active materials) (for example, antibodies or aptamers) are raised against the cytokines of interest to be measured. The cytokines of interest may include, Human IFNγ, Human TNFα, Human IL-1β, Human IL-4, Human IL-5, Human IL-6, Human IL-8, and Human IL-13. These detection antibodies or aptamers are tagged with element tags. Preferably, they are tagged in a covalent manner. For competitive assays, competition analytes (for example, cytokines) are tagged with elemental tags. Preferably, they are tagged in a covalent manner. It is desirable to tag each pool of antibody, aptamer, or cytokine with different elemental or isotopically enriched tags. For example, anti-Human IFNγ-Sm^{152}, anti-Human TNFα-Eu^{151}, anti-Human IL-1β-Dy^{164}, anti-Human IL-4-Eu^{153}, anti-Human IL-5-Tb^{159}, anti-Human IL-6-Pr^{141}, anti-Human IL-8-Sm^{154}, and anti-Human IL-13-Gd^{158} are prepared. To avoid tagging the active sites of aptamers due to their small size, it may be necessary to construct pre-labelled aptamer libraries, so that element-tagged aptamers are selected and produced based on their affinities to the respective cytokines. Optionally, if element-tags are not found to interfere with affinities, it may be easier to tag after the selection process. Both options are available. Alternatively, the kits may simply include a tag comprising a transition element with instructions for tagging the

biologically active material or competition analyte. Preparation of tags comprising transition elements are known to those skilled in the art.

2) Purification of element-tagged biologically active materials (for example, antibody, aptamer) or element tagged competition analytes (for example, cytokines). The element-tagged antibody, aptamer or cytokine is purified preferably using size exclusion chromatography, affinity chromatography, filtration, or dialysis.

3) Several supports (for example, columns, gels, plates, microwells and beads) have been designed for anchoring molecules (for example, analytes, aptamers or antibodies) through for example, the following mechanisms which include but are not limited to: (a) hydrophobic interactions, (b) hydrophilic interactions, (c) covalent reactions with amine and sulfhydryl groups, (d) avidin interactions with biotynlated antibodies, (e) Fc (crystallizable fragment of an antibody) affinities for Protein A or Protein G, or (f) through affinities for nickel or glutathione. Some examples of plates used for antibody attachment are Nunc MaxiSorp™, Pierce Maleic Anhydride™, Pall™ nitrocellulose and PVDF™ filter plates. Aptamer attachment may be achieved through several different methods, which includes but is not limited to: UV irradiation, covalent chemical bonds, or baking to glass, plastic, or membrane surface. Plates available include: Nunc CovaLink™ which use secondary amino groups to covalently bind 5' carboxyl group of DNA or RNA; and Nunc NucleoLink™ which covalently bind 5' phosphorylated DNA. Various filter-plates (Nunc™, Pall™) are available which are convenient for preparing 96 well bead

immunoassays simultaneously. Such filter-plates can be used in

conjunction with vacuum or centrifuge and preferably have a pore size $1/10^{th}$ of the size of the beads being used. Other options of bead containment are available and obvious to those skilled in the art and can be used to substitute filter-plates in all instances.

4) Coating of the microwell plates or beads with capture anti-cytokine antibodies or aptamers. This can be done for the sandwich affinity assays and the competitive affinity assays. In the sandwich assay, preferably a matched pair of antibodies or a matched pair of aptamers for each of the cytokines that are to be quantitated is designed. A matched pair of antibodies is two antibodies that recognize distinct epitopes on the cytokine so that a cytokine may be bound with high affinity to both antibodies simultaneously. The antibodies may be monoclonal antibodies or polyclonal antibodies. A matched pair of aptamers is similar, in that two aptamers are developed towards distinct epitopes on the cytokine of interest so that the cytokine is bound with high affinity to both aptamers simultaneously. In a sandwich assay, one of the matched pair of antibodies or aptamers is coated on the microwell plates or beads and serves to capture the target cytokine in the sample anchoring it to the surface. The second antibody or aptamer functions as a detection agent and is labelled with an elemental tag. The coating of microwell plates for multiple cytokine detection may be done by a) pipetting a mixture of the capture antibodies or aptamers into each well or b) by spotting different antibodies or aptamers separately using different tips (e.g. robotic). The advantage of a

spotting format is that the different capture antibodies or aptamers are

kept separate and many different custom plates can be produced for analyzing different combinations of cytokines such that production is easily designed by robotic application.

5) In sandwich assays and competition assays, after coating the solid supports with capture molecules, for example antibodies or aptamers to prevent non-specific binding, it may be necessary to passivate or treat the microwell plates or beads and filter-plate using a assay buffer prior to being used in the kit protocol. The assay buffer may contain sterile proteinaceous buffer (e.g. 1%BSA in 1xPBS with preservatives).

6) Components of Element-tagged Direct Affinity Assay kit for the simultaneous detection and quantitation of multiple cytokines. The kits may contain all or some of the following components: 1) tags comprising transition elements, 2) element-tagged biologically active materials (for example, antibodies or aptamers), 3) cytokine binding solid supports, for example microwell plate(s) or beads and filter-plate(s), 4) standard cytokines (which will be comparable to the National Institute for Biological Standards and Control (NIBSC) standards), 5) diluent buffers for re-suspending standard cytokines and element-tagged reagents, 6) wash buffers, 7) elution buffers and 8) assay buffers (optional for pre-treating plates), 9) instructions and protocols for direct affinity assay for the simultaneous detection and quantitation multiple analytes.

7) Components of Element-tagged Sandwich Affinity Assay kit for the simultaneous detection and quantitation of multiple cytokines. The kits may contain all or some of the following components: 1)tags

comprising transition elements, 2) element-tagged biologically active materials (for example, antibodies or aptamers), 3) pre-coated (with capture antibodies or aptamers) and pre-treated (with assay buffer) solid supports, for example microwell plate(s) or beads and filter-plate(s), 4) standard cytokines (which will be comparable to NIBSC standards), 5) diluent buffers for re-suspending standard cytokines and element-tagged reagents, 6) wash buffers, 7) elution buffers, and 8) assay buffers (optional for pre-treating plates), 9) instructions and protocols for sandwich affinity assay for the simultaneous detection and quantitation multiple analytes.

8) Components of Element-tagged Competitive Affinity Assay kit for the simultaneous detection and quantitation of multiple cytokines. The kits may contain all or some of the following components: 1) tags comprising transition elements; 2) element-tagged competition analytes (for example, cytokines), 3) pre-coated (with capture molecules, for example antibodies, aptamers or other analyte binding material eg. maleic anhydride groups or cytokine receptors) and pre-treated (with assay buffer) solid supports, for example microwell plate(s) or beads and filter-plate(s), 4) standard cytokines (which will be comparable to NIBSC standards), 5) diluent buffers for re-suspending standard cytokines and element-tagged reagents, 6) wash buffers, 7) elution buffers and 8) assay buffers (optional for pre-treating plates). 9) Protocols and instructions for the competitive affinity assay.

Optionally, the solid supports can bind the analyte and competition analyte directly.

[0209] The kits may also include instructions or protocols for carrying out the assays. The following are some sample protocols:

9) Protocol for Element tagged Direct Affinity Assay kit for the simultaneous detection and quantitation of multiple cytokines.

a.) Prepare purified standard cytokines (which will be comparable to NIBSC standards) by adding a prescribed volume of standard cytokine diluent buffer (e.g. 1%BSA in 1xPBS) into each vial, allowing to dissolve for 5 minutes at room temperature, and inverting several times. Prepare serial dilutions (e.g. 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, 15.6pg/ml, 7.8pg/ml, 3.9pg/ml, and 0pg/ml).

b.) Prepare the sample containing cytokines of interest (eg. patient EDTA-plasma samples) by thawing, mixing and filtering if necessary.

c.) If beads are being used, beads must be suspended and diluted accordingly. Aliquote 100µl of beads into each microwell of filter plate.

d.) Pipette an aliquote (eg. 100µl) of either sample or serially diluted standard into each well (in duplicate or triplicate as desired). Shake on orbital shaker at room temperature for 1-2hrs.

e.) Aspirate (or with beads and filter-plate use centrifuge or vacuum) and wash 3 times with 400µl of wash buffer (e.g. 1%BSA in 1xPBS).

f.) Add an aliquote (eg. 100μl) of element-tagged biologically active materials (for example, antibodies or aptamers) at appropriate dilution (in detection diluent buffer; e.g. 1%BSA in 1xPBS). Shake on orbital shaker at room temperature for 1-2hrs.

g.) Aspirate (or with beads and filter-plate use centrifuge or vacuum) and wash 3 times with 400μl of wash buffer (e.g. 1%BSA in 1xPBS).

h.) Aspirate (or with beads and filter-plate use centrifuge or vacuum) plate.

i.) Add 50-100μl elution buffer containing an acid solution with elemental spike, preferably an element that is soluble in the acid and close to the same atomic mass as the elements to be measured (e.g. 3% HCl, 1ppb Pr). Shake on orbital shaker at room temperature for 5-10 minutes.

j.) Measure and quantitate elements of interest using atomic mass or optical spectrometer, preferably ICP-MS.

10) Protocol for Element tagged Sandwich Affinity Assay kit for the simultaneous detection and quantitation of multiple cytokines.

a.) Prepare purified standard cytokines (which will be comparable to NIBSC standards) by adding a prescribed volume of standard cytokine diluent buffer (e.g. 1%BSA in 1xPBS) into each vial, allowing to dissolve for 5 minutes at room temperature, and inverting several times. Prepare serial dilutions (e.g. 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, 15.6pg/ml, 7.8pg/ml, 3.9pg/ml, and 0pg/ml).

b.) Prepare the sample containing cytokines of interest (eg. patient EDTA-plasma samples) by thawing, mixing and filtering if necessary.

c.) If beads are being used, beads must be suspended and diluted accordingly. Aliquote 100µl of beads into each microwell of filter plate.

d.) Pre-treat microwell plate or beads and filter-plate with 400µl of assay buffer (e.g. 1%BSA in 1xPBS) per well. Shake on orbital shaker at room temperature for 1-2hrs. (optional, may not be necessary)

e.) Pipette an aliquote (eg. 100µl) of either sample or serially diluted standard into each well (in duplicate or triplicate as desired). Shake on orbital shaker at room temperature for 1-2hrs.

f.) Aspirate (or with beads and filter-plate use centrifuge or vacuum) and wash 3 times with 400µl of wash buffer (e.g. 1%BSA in 1xPBS).

g.) Add an aliquote (eg. 100µl) of element-tagged biologically active materials (antibodies or aptamers) at appropriate dilution (in diluent buffer; e.g. 1%BSA in 1xPBS). Shake on orbital shaker at room temperature for 1-2hrs.

h.) Aspirate (or with beads and filter-plate use centrifuge or vacuum) and wash 3 times with 400µl of wash buffer (e.g. 1%BSA in 1xPBS).

i.) Aspirate (or with beads and filter-plate use centrifuge or vacuum) plate.

j.) Add 50-100µl elution buffer containing an acid solution with elemental spike, preferably an element that is soluble in the acid and close to the

same atomic mass as the elements to be measured (e.g. 3% HCl, 1ppb Pr). Shake on orbital shaker at room temperature for 5-10 minutes.

k.) Measure and quantitate elements of interest using atomic mass or optical spectrometer.

11) Protocol for Element tagged Competitive Affinity Assay kit for the simultaneous detection and quantitation of multiple cytokines.

a.) Prepare purified standard cytokines (which will be comparable to NIBSC standards) by adding a prescribed volume of standard cytokine diluent buffer (e.g. 1%BSA in 1xPBS) into each vial, allowing to dissolve for 5 minutes at room temperature, and inverting several times. Prepare serial dilutions (e.g. 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, 15.6pg/ml, 7.8pg/ml, 3.9pg/ml, and 0pg/ml).

b.) Prepare the sample containing cytokines of interest (eg. patient EDTA-plasma samples) by thawing, mixing and filtering if necessary.

c.) If beads are being used, beads must be suspended and diluted accordingly. Aliquote 100µl of beads into each microwell of filter plate.

d.) Pre-treat microwell plate or beads and filter-plate with 400µl of assay buffer (e.g. 1%BSA in 1xPBS) per well. Optionally, shake on orbital shaker at room temperature for 1-2hrs.

e.) Pipette an aliquote (eg. 100µl) of either sample or serially diluted standard into each well (in duplicate or triplicate as desired). Shake on orbital shaker at room temperature for 1-2hrs.

f.) Aspirate (or with beads and filter-plate use centrifuge or vacuum) and wash 3 times with 400µl of wash buffer (e.g. 1%BSA in 1xPBS).

g.) Add an aliquote (eg. 100µl) of element-tagged competition analyte (for example, cytokines) at appropriate dilution (in diluent buffer; e.g. 1%BSA in 1xPBS). Shake on orbital shaker at room temperature for 1-2hrs.

h.) Aspirate (or with beads and filter-plate use centrifuge or vacuum) and wash 3 times with 400µl of wash buffer (e.g. 1%BSA in 1xPBS).

i.) Aspirate (or with beads and filter-plate use centrifuge or vacuum) plate.

j.) Add 50-100μl elution buffer containing an acid solution with elemental spike, preferably an element that is soluble in the acid and close to the same atomic mass as the elements to be measured (e.g. 3% HCl, 1ppb Pr). Shake on orbital shaker at room temperature for 5-10 minutes.

k.) Measure and quantitate elements of interest using atomic mass or optical spectrometer.

[0210] The kits may include two or more biologically active materials having distinguishable elemental tags for simultaneous determination of two or more analytes. In the case of kits for competition assays, the kits may include two or more competition analytes having distinguishable elemental tags and corresponding two or more capture molecules for the simultaneous determination of two or more analytes. Finally, the kits may also include a combination of components for the direct affinity assay, the sandwich affinity assay and the competitive affinity assay. For example, a tagged competition analyte is used to measure analyte A by a competition affinity assay

and two differently tagged biologically active materials are used to measure analytes B and C by a direct affinity assay.

Example 20. Cytokine Immunoassay

[0211] The following provides an example of the methods of the invention using element tags to determine the concentration of an analyte (eg. cytokine) of interest in a complex sample (Figures 6-11). Either ~~Nanogold~~ NANOGOLD-Fab'™ or another element-labeled Fab' (or other biologically active material) that will bind specifically to the target analyte (or primary, secondary, or tertiary antibody) may be used. There are three types of immunoassays that may be used:

a)Direct immunoassay, which would involve trapping the protein(s) (Human IgG, FLAG-BAP, Figure 6) or cytokine(s) of interest (Human Interferon gamma, IFN-g; Human Interleukin 5, IL-5, Human Interleukin 6, IL-6, and Human Interleukin 8, IL-8, Figure 7) using a specified solid support such as microwell plates or microspheres (Maleic Anhydride microwell plates were used in experiment shown in Figure 6 and Pall NT Acrowell Filter microwell plates were used in the experiment shown in Figure 7), incubating with an excess of element-tagged (anti-Human IgG Fab'-nanoAu, Figure 6; anti-IFN-γ-Sm, anti-IL-5-Eu, anti-IL-6-Tb, and anti-IL-8-Dy, Figure 7) or untagged primary antibody or other biologically active material (anti-BAP, Figure 6) specific to the target analyte, washing off the un-reacted antibody, adding element-tagged secondary antibody (anti-mouse-Eu, Figure 6), washing off un-reacted antibody and then subjecting the antibody-analyte complexes to atomic mass or optical spectrometry (eg. ICP-MS).

b)Sandwich immunoassay which would involve trapping the cytokine(s) of interest (IL-6 in Figure 8; IL-6 and IL-8 in Figure 9; IL-6, IL-8, IFN-γ and TNF-α in Figure 10; and IL-6 and TNF-α in Figure 11) by incubating the sample containing the cytokine(s) of interest with a specified solid support such as the microwell

plates (Figures 8-10) or microspheres (Figure 11) that have been pretreated (bound) with an excess of primary antibody or biologically active material specific to the target analyte. The microwell plates or microspheres are washed to remove the un-reacted portion of the sample, and the cytokine is then exposed to element-tagged or untagged biologically active material (eg. primary antibody), washed to remove unreacted biologically active material, and if necessary incubated with element-tagged secondary antibody. The washed cytokine complexes are then subjected to atomic mass or optical spectrometry (eg. ICP-MS).

c) Competition immunoassay, which would involve trapping the target cytokine of interest (protein X) by incubating a specified solid support (eg. microwell plates or microspheres which may or may not be pretreated with an excess of primary antibody or biologically active material specific to the target analyte), adding the analyte-containing sample, washing off the unbound components, and then exposing the specified support with antibody-protein X complexes to a known amount of purified element-labeled protein X. The washed cytokine complexes are then subjected to atomic mass or optical spectrometry (eg. ICP-MS).

[0212] PBS buffer A is prepared as follows: 150mM NaCl; 20mM phosphate, pH 7.4; 1% BSA (bovine serum albumin). All eppendorf tubes, micro-titer plates, and filters, and slurry of microspheres to be used subsequently are treated with PBS buffer A for 1 hour at room temperature to block non-specific binding. This treatment will reduce the non-specific interactions that occur between the plastic used and the analyte and ~~Nanogold~~ NANOGOLD-Fab'™. Alternatively low retention plastic products can be used (e.g.

Axygen tubes). Following this a solution of the analyte (cytokine, peptide, protein, etc.) in a specified concentration range (eg. 1-100ng/ml in Figure 6; 0.1-10ng/ml in

Figure 7; 3.12-300pg/ml IL-6 in Figures 8, 9 and 10; 31.2-2000pg/ml IL-8 in Figure 9 and 10; 15.6-1000pg/ml of IFNγ and TNF-α in Figure 10), 16-10,000pg/ml of TNF-α and IL-6 in Figure 11) in PBS buffer A or biological matrix (eg. EDTA plasma) is prepared. All dilutions are stored on ice. Subsequently, 100ul of analyte or either PBS buffer A or other biological matrix such as EDTA-plasma (for negative controls) is pipetted into the individual wells of the micro-titer plate (or set of eppendorf tubes). If using ~~Nanogold~~ NANOGOLD-Fab'™, to remove large unbound gold particles, the ~~Nanogold~~ NANOGOLD-Fab'™ is pre-filtered through 300KDa MICRCON™ (or ~~Centricon~~™ CENTRICON) centifugal filter devices. Filtered ~~Nanogold~~ NANOGOLD-Fab'™ (and other element-tagged and untagged biologically active materials, eg. in Figure 6, anti-Human IgG-Fab'-nanoAu, anti-BAP, anti-mouse-Eu; in Figure 7, anti-IFN-γ-Sm, anti-IL-5-Eu, anti-IL-6-Tb, and anti-IL-8-Dy; in Figure 8, anti-IL-6-Tb; in Figure 9, anti-IL-6-Tb, anti-IL-8-Dy; in Figure 10, anti-IFN-γ-Sm, anti-TNF-α-Eu, anti-IL-6-Tb, and anti-IL-8-Dy; and in Figure 11, anti-TNF-α-Eu and anti-IL-6-Tb) required are diluted in PBS buffer A or other biological matrix such as EDTA-plasma are prepared as follows: a 1:50 dilution is produced by adding 60μl of ~~Nanogold~~ NANOGOLD-Fab'™ in 2940μl PBS buffer A. A 1:500 dilution is then produced by adding 100μl of 1:50 ~~Nanogold~~ NANOGOLD-Fab'™ to 900μl of PBS buffer A. In methods a) and b) depending on concentration range of analyte, 100 to 500 μl of 1:500 (or other suitable dilution) of ~~Nanogold~~ NANOGOLD-Fab'™ (or other required element-tagged or untagged biologically active materials) is then added to the wells of the plate and then incubated for 1-2 hours at room temperature. In method c) an element-tagged analyte (cytokine) is added to each sample. In methods a-c) microwell plates are washed by pipetting fresh wash buffer in and out of wells 1-3 times. Microspheres

in filter plates are washed through centrifugation of wash buffer (1-3 times).

Microspheres in tubes are washed through centrifugation of wash buffer (1-3 times). Preferably all procedures are at 4C. If an untagged biologically active material has been used in the proceeding step, the sample may be incubated with an element-tagged biologically active material (eg. secondary antibody) to bind the cytokine complex. The wash step will then need to be repeated. If an untagged biologically active material has been used in the proceeding step, the sample may be incubated with an element-tagged biologically active material (eg. tertiary antibody) to bind the cytokine complex. . The wash step will then need to be repeated. Finally, a fixed volume of 10%HCl/1ppbIr is added to each well. Ir and/or Ho provides an internal standard for ICP-MS quantitation and the acid solution is suitable for the elemental analysis.

[0213] Experimental results obtained according to method a) are given in Figures 6 and 7, using Human IgG and FLAG-BAP (Figure 6) and IFN-γ, IL-5, Il-6, and IL-8 (Figure 7) as the analytes of interest with anti-Human IgG F'ab-Au, anti-BAP and anti-mouse-Eu (Figure 6), and anti-IFN-γ-Sm, anti-IL-5-Eu, anti-IL-6-Tb, and anti-Il-8-Dy as the (un)tagged antibody (Figure 7). Experimental results obtained according to method b) are given in Figures 8-11.

[0214] In Figure 6, Maleic Anhydride microwell plates (Pierce) were used to anchor the proteins to the bottom of each of the 96 wells. Wells in triplicate contained concentrations of both Human IgG and FLAG-BAP in either 0, 1, 10 or 100ng/ml in PBS buffer A. According to method a) Human IgG was bound with anti-Human IgG Fab'-nanoAu and FLAG-BAP was bound with anti-BAP and anti-mouse-Eu antibodies. Detection and quantitation was obtained using an ICP-MS.

[0215] In Figure 7, Pall Acrowell filter plates were used to anchor the proteins to the bottom of each of the 96 wells. Wells in triplicate contained concentrations of both IFN-γ, IL-5, IL-6, and IL-8 in either 0, 0.1, 1, or 10ng/ml in PBS buffer A and according to method a) these human cytokines were bound with anti-IFN-γ-Sm, anti-

IL-5-Eu, anti-IL-6-Tb, and anti-IL-8-Dy antibodies respecitively. Detection and quantitation was obtained using an ICP-MS.

[0216] In Figure 8-10, ~~Quantikine~~ QUANTIKINE microwell plates (R&D systems) were used to anchor the proteins (cytokines) to the bottom of each of these 96 wells. Wells in triplicate contained concentrations of (as shown in graphs as well as 0pg/ml for negative control) in PBS buffer A (or EDTA-plasma) and according to method b) these human cytokines were bound with specific element tagged antibodies (see Figures 8-10). Detection and quantitation was obtained using an ICP-MS.

[0217] In Figure 11, Fluorokine microspheres (R&D systems) were used to anchor the proteins (cytokines) and supplied filter plates were used to contain and perform each assay. Wells in triplicate contained concentrations of (as shown in graphs, as well as 0pg/ml for negative control) in PBS buffer A and according to method b) these human cytokines were bound with specific element tagged antibodies (see Figure 11). Detection and quantitation was obtained using an ICP-MS.

[0218] While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

[0219] All publications, patents, and parent applications are herein incorporated by reference in their entirety to the same extent as if each individual

publication, patent or parent application was specifically and individually indicated to be incorporated by reference in its entirety.